A Growing Problem: The Missing Link for Ecological Success by the Invasive *Avrainvillea*

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Abstract

The invasive green alga *Avrainvillea amadelpha* (Montagne) A. Gepp & E.S. Gepp (*Avrainvillea*) has a highly unusual anatomy – typical of its siphonous construction; there are no crosswalls that separate cells within the body of this plant. First detected in Hawaiʻi in deep waters in 1981 (Brostoff 1989), *Avrainvillea* has readily spread as a monoculture up to shallow reefs and along the south shore of Oʻahu, displacing diverse communities of native reef plants and seagrass. Such a rapid spread and unusual body plan lead to questions of the relationship between plant body and ability to sequester essential nutrients.

In this study, I assessed the differences in the morphology of *Avrainvillea*, specifically the balance between upper and basal portions as well as differences in nitrogen (N) content and potential N-sources between the basal holdfast and the upright blades. Intra-plant sampling was conducted by collecting specimens from both offshore and nearshore sites in Maunalua Bay, Oʻahu, where a field of invasive algae spans an approximately 1.5km^2 area. Additionally, N content of groundwater beneath dense patches of *Avrainvillea* was compared to the N content of groundwater in nearby areas with no algae to assess if *Avrainvillea* draws down this potential source of nutrients as a second test of this plant's impact. Tissue samples were analyzed for % inorganic nitrogen and δ^{15}N values, while groundwater samples were analyzed for concentrations of dissolved inorganic nutrients. These results demonstrated a significantly higher concentration of N in the upright blades compared to the basal holdfasts for the same plant. In contrast, δ^{15}N levels tended to be higher in the basal holdfasts, suggesting the nutrient partitioning occurred. Finally, in about half of the groundwater nutrients samples, areas without algae were substantially higher than in locations with algae. Overall, these studies begin to give insight in the competitive strategies that have allowed *Avrainvillea* to dominate native plant communities.

Keywords: invasive marine plant, loss of biodiversity, marine ecology, resource management

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Chapter One: Literature Review

Management of invasive species has become a severe issue over the last five decades (Pimentel *et al.* 2005, Lowe *et al.* 2000). Hawai‘i currently hosts more than 100 invasive species today, both terrestrial and aquatic (dlnr.hawaii.gov/hisc/info/invasive-species-profiles/). The isolation of Hawai‘i from continental landmass by approximately 3,700 km allowed for a highly endemic biota, levels of which have decreased in the Main Hawaiian Islands (MHI), but can still be seen in the Northwestern Hawaiian Islands (NWHI) (Kane *et al.* 2014). This decrease can be tied to a number of factors, notably human population increases in coastal communities, and the subsequent urbanization of watersheds, sedimentation, increase in fishing, and other anthropogenic impacts (Williams *et al.* 2015).

The competition for space between corals and algae is considered a fundamental component to the status and health of reefs (McCook *et al.* 2001). Environmental and anthropogenic impacts can shift the dominant organism on reefs. The relative-dominance paradigm, first presented by Littler and Littler (1984) suggests four main groups of sessile photosynthetic organisms will dominate on reefs as a function of long-term nutrient levels and herbivore activity: corals, turf algae, crustose coralline algae, and frondose macroalgae (Figure 1.2). Further, certain algae – the crustose corallines – are critical to recruitment of reef-building corals, giving off chemical signals the corals use to determine if an area is viable for settlement or not (Richmond 1997). Algae are usually found growing in ecosystems where there is a high input of light, water motion, and nutrients. Optimal combinations of irradiance, nutrients, and low grazing pressures can lead to algal dominance on a reef. However, nutrient-rich waters relieve macro-limiting factors for plant growth, and if nutrients are artificially increased, algal blooms are a typical outcome (Amato *et al.* 2016).

With highly competitive species, algal growth and dispersal is quick and largely successful. In the genus *Bryopsis*, fragments as small as a millimeter across can successfully settle and grow to new adult plants (Vroom and Smith 2001). Because they can reproduce via fragmentation, this gives the algae of Bryopsidales a significant advantage over other types of algae. Whereas storms and crashing waves often can cause harm to many algae by smashing them into many smaller fragments, Bryopsidalean algae benefit by spreading their smashed pieces and growing new adults from these fragments. Another invasive algae in the order Bryopsidales is *Caulerpa taxifolia*, known commonly as “killer algae” as a result of its incredible ability to invade an area, crowd out native seagrasses, and potential to be highly toxic to herbivores (NIMPIS 2010).

In 1981 when *Avrainvillea* was first detected in Hawaiian waters, it was identified as *Avrainvillea amadelpha* (Brostoff 1989), but this name has since become contested and is in the process of genetic identification at the University of Hawai‘i at Mānoa (Rachel Wade, personal communication). I will refer to this alga as *Avrainvillea* from here. As one of the five most invasive species in Hawai‘i, the adult morphology of *Avrainvillea* has been initially characterized (Smith *et al.* 2002; Vroom *et al.* 1998), but except for the work of Kimberly Peyton at Maunalua Bay (Peyton 2009), there is little other information on the ecology of the species in Hawai‘i. As a perennial, the adult forms can live for long periods. Although it is a slow growing plant, as a perennial, it can outlive other more short-lived species, giving *Avrainvillea* an additional advantage over native species (Peyton 2009).

Avrainvillea is in the order Bryopsidales, a classification of multinucleate siphonous green macroalgae exhibiting heteromorphic sporic meiosis, wherein there are two free-living stages, one of which is a gamete-producing phase and one a spore-producing phase, that are morphologically different from each other. More derived members of the Bryopsidales order reproduce via gametic meiosis, with a single free-living form producing gametes, or most commonly, through asexual reproduction via fragmentation, where an individual can reproduce asexually by breaking apart and regrowing (Vroom & Smith 2001). Other *Avrainvillea* species in the Caribbean have been characterized more thoroughly (Littler and Littler 1992; 2004). The Caribbean species exhibit sporic meiosis, though no reproductive structures were ever present in specimens or described (Littler & Littler 1992).

Compared to other algae that reproduce at their apical cells, usually towards tips of the algae, the basal holdfast of *Avrainvillea* is considered to be more regenerative than the blades (Littler & Littler 1999). The elasticity in the morphology of the basal holdfast also allows for it to grow in soft sediments of sandy or muddy areas, as well as on rocks (Littler & Littler 1992). Because of this, it is suited for many different environments and becomes difficult to remove once it has been introduced into an area. These basal holdfasts often take much of the sediment with them when picked, which can cause changes in the structure of the substrate during removal and conservation efforts (Longenecker *et al*. 2011).

Previous research has studied the chemical cause behind the lack of predatory herbivores that feed on other species of *Avrainvillea*. *Avrainvillea erecta* studied in Nigeria was found to secrete a chemical called avrainvilleol, which was seen to deter fish and other marine organisms, and makes up approximately one percent of the dry mass of the plant (Chai *et al.* 2015). It's likely this chemical is one reason *Avrainvillea* was found to be one of the algae least affected by feeding when compared to other seaweeds (Hay *et al.* 1990). Two of the few natural grazers of this alga are the gastropods of the *Costasiella* genus and the crab *Thersandrus compressus*, found in Belize, which feed on *Avrainvillea longicaulis*.

Research on *Avrainvillea* continues in Hawai‘i with increasing urgency as the alga displaces native species and spreads in Hawaiian waters. Once *Avrainvillea* inhabits an area, it often acts as a substrate for other invasive algae to colonize the area. It’s been suspected that this alga has been a source of competition for the Hawaiian native seagrass, *Halophila hawaiiana* (Smith *et al.* 2002; Peyton 2009) as well. When compared to other non-native algae such as *Acanthophora spicifera*, *Gracilaria salicornia*, and *Hypnea musciformis*, *Avrainvillea* showed the lowest potential to reproduce via fragmentation, with the highest success of growth for *Avrainvillea* found in the largest fragment size at 3 cm (Smith *et al.* 2002). Although *Avrainvillea* showed a low potential to reproduce via small fragmentation, it is incredibly invasive, which raises more questions about whether fragmentation is the primary form of reproduction, what common sizes of fragments are most often the cause of spreading, or if there’s another unknown aspect affecting the success of *Avrainvillea*.

In Maunalua Bay, urbanization of the area has brought with it excess sedimentation, disturbance or destruction of habitats, and a marked decline in the number of fish in the nearshore marine environment, (Atkinson 2007). Compared to other locations around O‘ahu, the reef ecosystem of Maunalua Bay is considered impaired by anthropogenic affects (Rodgers *et al.* 2009), which is especially clear in the Paikō Peninsula area, adjacent to the urbanized Hawaii Kai Marina (Figure 2.1). The sandy peninsula is naturally occurring and surrounds a wetland fed by a freshwater spring (Shallenberger 1977; Stump 1981; Lassalle 2003). Springs like this in other parts of Maunalua Bay bring freshwater high in nitrogen into the sediments where the roots of *Avrainvillea* are growing (Richardson *et al.* 2016). Paikō Lagoon is now a wildlife sanctuary, but was in the early 20th century was a privately-owned fish pond (Figure 1.1). In 1972 the lagoon was dredged despite Division of Fish and Game objections to the removal of shorebird and shore crab habitat (Lum 1978); in 1974, the area was designated as a bird sanctuary (Komoto and Gombos 2007). Maunalua Bay, including the ocean-side of Paikō Peninsula, has one of Hawaii's most shallow and broad fringing reefs (Fletcher 2005), once characterized by meadows of the seagrass *Halophila hawaiiana* (Peyton 2009; Unabia 1984). The area is now composed primarily of invasive algae, especially *Avrainvillea*.

In the early part of 2009, as part of the American Recovery and Reinvestment Act, also known as the 2009 stimulus package, $3.4 million was spent to clear this and other invasive algae out of Maunalua Bay. Over 23 of 54 acres were successfully cleared by hand by volunteers in 7,000 man hours ("The Great Huki Project"). In less than six years, the algae have grown back and covers the area once more (Figure 2.5). History has shown time and time again that with this and other invasive species, even a great deal of money is not sufficient to address their impacts if it is spent without a basic understanding of the fundamental biological characteristics of the species.

Avrainvillea was first detected in deep waters off Oʻahu in 1981 (Brostoff 1989). Since then, it has spread vertically to tidal reefs such as those in Maunalua Bay, and horizontally along the south shore of Oʻahu. In May 2017 during the Smithsonian-sponsored MarineGEO surveys, a large infestation was detected well outside its usual range, on the east side of Oʻahu in Kāneohe Bay at Mokoliʻi, commonly known as Chinaman's Hat (Celia Smith, personal communication). With the broadest known depth range of any algae in Hawaiʻi, *Avrainvillea* is found in waters one third of a meter deep such as the reef accessible via Paikō Peninsula, to 80 meters offshore of Ewa, O‘ahu. Very little data currently exists characterizing the ecology of *Avrainvillea* in Hawai‘i even though it presents an increased ecological threat to Hawaii’s reefs. This genus is known for its unique morphology, in that it has two prominent components; a basal holdfast that grows under the sediment, and one to numerous upright photosynthetic blades of varying sizes that stand upright in the water column. This alga grows in mounds, trapping sediments in both the basal holdfasts and the dense bladed regions, changing the water flow and the underwater landscape, thus creating oxygen-starved benthic environments (Littler *et al.* 2004). The morphology of *Avrainvillea* might allow this seaweed a significant competitive advantage, making this alga a serious threat to the biodiversity of Hawaiian reefs. For such a widespread and pressing invasive species, there’s a striking gap in knowledge.

Figure 1.1. A historical look at Paikō Peninsula and Lagoon, circa 1925 (Hawaii State Archives).

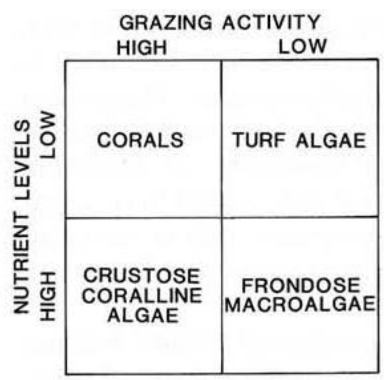

Figure 1.2. A diagram representing the relative dominance paradigm from Littler *et al.* (1991).

Chapter Two: Nutrient Partitioning and Uptake in *Avrainvillea*

Introduction

This study was designed to investigate variation the size ratio of the basal holdfast to the blades of *Avrainvillea*, as a way to evaluate an understudied part of the ecology of a dominant invasive. Because photosynthesis is a crucial process leading to plant growth, if an organism can provide more surface area for sunlight to come into contact with chloroplasts, more energy can be created. However, in many cases, algal growth is nitrogen limited (Smith 1984), and if an alga can access otherwise untapped 'in-ground' nitrogen as an additional nutrient source, a larger basal holdfast to access more nutrients could be a successful strategy, even though this strategy encumbers added costs of below-ground tissues.

Localized nutrients in algal tissue

Because *Avrainvillea* is siphonous and therefore has no cellular segmentation commonly found in other plants (Vroom and Smith 2011), the values of stable isotopes of nitrogen, a powerful indicator - the $\delta^{15}N$ parameter - should be equal for tissues found in the base and blade of this plant (Vroom *et al.* 1998). However, as the base is considered more regenerative than the photosynthetic blades (Smith *et al*. 2002), it is also possible that the bulk of the nutrients acquired by the plant are more readily stored in the base, to provide for rapid growth. This could skew the ^{13}C:^{12}C and ^{15}N:^{14}N ratios detected in tissue analysis. Further, the base may acquire nutrients from sediments that could have a different signal than that seen from water column nutrients in upright fans. Localization of nutrients within *Avrainvillea* may be an adaptive strategy for ecological success. Discovering whether there is a partitioning in nitrogen concentration between the basal holdfast and blades could help support the importance of removing the whole plant from affected areas to slow regrowth.

Groundwater nutrients

Submarine groundwater discharge (SGD) can be a conduit for the transport of land-sourced nitrogen and phosphorus to coastal environments, and in many cases is comparable to surface water contributions (Richardson *et al.* 2016). Continuous nitrogen loading of coastal waters via SGD has been shown to promote phase-shifts in nearshore reef flats, supporting the existence of a relative-dominance paradigm (Figure 2.8). The shift at Paikō from *Halophila hawaiiensis* meadows to dominance by *Avrainvillea* would suggest the existence of nutrient loading, as *Halophila hawaiiensis* is as competitive if not more than *Avrainvillea*, so long as the water quality is good (Peyton 2009).

The reef at Paikō is a prime site to study this alga because although this area is not as well characterized compared to other areas of Maunalua Bay in respect to groundwater discharge (Kelly *et al.* 2013; Richardson *et al.* 2016; Bishop *et al.* 2017), Paikō has the largest continuous field of *Avrainvillea* on any reef on Oʻahu. This allows for numerous locations to examine and test bases for this alga's expansive and invasive growth. By comparing nitrogen concentrations in the groundwater with the nitrogen concentrations in the algal tissue, a relationship may be found between the amount of nitrogen the algae are accessing for nutrients and the levels of nitrogen in areas where there are no algae. Groundwater in carbonate sand generally has a high concentration of nutrients available to plants that are able to access it, likely allowing them to continue sustained growth in the absence of nutrients in the water column, or grow unchecked for a long period of time (Dailer *et al.* 2010).

Nearshore and offshore differences in water and pore water sources

Because the reef at Paikō is continuous from a meter at the shore horizontally several hundred meters to the reef crest, this provides a good location for a study site to test for the difference in nearshore and offshore changes. If this subsection of the reef has similar SGD as reported for much of Maunalua Bay (Richardson *et al.* 2016), the nearshore water samples should have higher values of dissolved inorganic nitrogen and dissolved inorganic phosphorus than the algae-free sediment sites. Similarly, the algae tissue samples from nearshore sites should have a higher percentage of nitrogen and possibly higher $\delta^{15}N$ concentrations than offshore sites.

Significance

Non-indigenous Invasive Species (NIS) are threatening local environments and economies around the world. Field work by Kimberly Peyton during her dissertation research in Botany, UHM (Peyton 2009) established *Avrainvillea* as one of the most invasive algal species in Hawaiʻi and its detrimental impact on Hawaiian coastal diversity, environmental landscape, and ultimately on the economy (Vroom and Smith 2011; Davidson *et al.* 2015). Algal blooms for one main Hawaiian island alone cost the County of Maui over \$20 million year^{-1} in lost revenue (Cesar & Beukering 2004) for one of five well-known NIS. Because there is such an extensive gap in knowledge about *Avrainvillea*, it poses significant challenges for resource managers and is a threat to fisheries and live corals. Currently, the only method of controlling the invasion of *Avrainvillea* is to remove it by hand, as is continuously done through algal cleanups run by the Botany at UHM, Mālama Maunalua, the Nature Conservancy, and the Waikīkī Aquarium, where community members are welcome to join together to clear invasive algae from Oʻahu's reefs.

With little to no grazing pressure in Hawai‘i on this plant, understanding fundamental biological characteristics of *Avrainvillea* can begin to build the base for future studies and conservation efforts to preserve and maintain the remarkable diversity of Hawaiian reef ecosystems.

Materials and Methods

Site Description

Avrainvillea was selected for this study because of its unique morphology and invasiveness on O‘ahu. Maunalua Bay, specifically the ocean side of Paikō Peninsula, was chosen as the location of this study because of a large field of *Avrainvillea* intermixed with other algae, approximately 400 m in length along the beach, and begins in some places a meter from shore to an average of 200 m offshore (Figure 2.1). The area at Paikō is also the site of many algae cleanups by Mālama Maunalua (Longenecker 2011), and although a concerted effort began in 2009 for The Great Huki (http://www.fpir.noaa.gov/HCD/hcd_maunalua.html), most of the cleared patches have grown back. Fifteen sample sites were chosen in two categories, eight along the nearshore border, and seven on the offshore border, at even intervals along a transect line. Site four was dominated by dense mats of *Gracilaria salicornia*. Even after removal of these mats at site four, no *Avrainvillea* could be identified within a three meter range of the original designated site, and therefore no samples were taken. Collections occurred over a period of three days, always in the morning from 6:30am-12:00pm, starting during low tide.

Sampling strategies

Once the algae samples were collected, they were immediately transported to the University of Hawai‘i at Mānoa and cleaned to free plant parts of epiphytes as well as sand. The *Avrainvillea.* samples had other algae attached, *Gracilaria salicornia* and *Acanthophora spicifera* being the most common, along with sponges, snails, and other invertebrates. Sediments and sand also needed to be removed, particularly from the basal holdfasts of the samples. The samples were cleaned to a point where no more foreign material could be removed without greatly impacting the sample itself.

Once cleaned, samples were split into basal holdfasts and blades. The separation point was determined by cutting at the base of the stalk, at the point where the blade begins to branch out of the basal holdfast. Additionally, the basal holdfast sits under the sediments and oftentimes has both a different coloring and texture to the upright blades and stalks. Sorted samples were then placed to dry in an oven for 10 days at 60°C to a constant weight. Once dried, samples were cleaned further, removing any missed sediments or other small shell and rock pieces.

Dried tissues were crushed with a mortar and pestle to a homogenous fine mixture, 0.2-0.6 µg of which were placed in Eppendorf tubes and sent to the SOEST Biogeochemical Stable Isotope facility for $\delta^{13}C$ and $\delta^{15}N$ analysis. These were calculated as a ratio of ^{13}C:^{12}C and ^{15}N:^{14}N, and calculated with (Peterson and Fry 1987):

$$\delta X = [(R_{sample}/R_{standard})-1] \times 10^3$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio.

A random number generator was used to select 20 of the 30 samples, 10 from the nearshore sites and 10 from the offshore sites. The remaining samples were kept so they may be used once more funding becomes available. This project is a geographical survey of Paikō Peninsula and an exploratory first step in understanding the ecology of *Avrainvillea*, and with more funding and time for processing samples, replicates should be taken at each site to confirm the trends seen in this project for a higher accuracy.

Porewater sampling

Porewater samples were also taken at all 15 sites and analyzed for dissolved phosphorus and nitrogen (TP and TN). This tested for the difference in nitrogen and phosphorus in the groundwater, to evaluate whether *Avrainvillea* might be accessing the nitrogen nutrients in the groundwater that other algae do not have access to without such basal holdfasts as *Avrainvillea*.

Porewater collectors created from aquarium stones attached to 0.75 meter of airline tubing were inserted 5 cm into the sediment at each site, one as close as possible to or within the sampled algae patch, and one in a sandy spot clear of algae. The airstone was assumed to take in water in a spherical area estimated at a 2.5 cm radius, and therefore should not be taking in any surface water (Larned 1997). The airstone and tubing were left for approximately 15 minutes after being inserted to allow sediments to stabilize, after which a 60 mL syringe with a 0.25 mm, 0.45 µm filter was attached to the tubing. Initial 20 mL samples were collected and discarded to ensure sample quality, by removing possible contamination from the water column (Larned 1997).

Water chemistry

Approximately 120 mL was then collected at each site and placed into clean containers and transported to campus, where they were immediately frozen until they could be sent to the SOEST Lab for Analytical Biogeochemistry. There, a Seal Analytical AA3 HR Nutrient Autoanalyzer determined ammonium, nitrate and nitrite, and silicate concentrations. Ammonium was measured by reacting the sample with o-phthalaldehyde (OPA) at 75°C to form a fluorescent species in the presence of a borate buffer and sodium sulfite in a quantity to the ammonium concentration, following methods set by Kerouel and Aminot (1997) (S-Lab). Nitrate and nitrite were measured through the diazo reaction set forth by Armstrong *et al.* (1967) and Grasshoff *et al.* (1983), by reducing nitrate to nitrate via a copper-cadmium reductor column and reacting the nitrite with sulfanilamide under acidic conditions, forming a diazo compound. This compound couples with N-(1-Naphthyl)ethylenediamine dihydrochloride, forming a purple dye, which is used to then determine the concentration of nitrite colorimetrically at 550 nm. Silicate concentration was determined colorimetrically at 550 nm after a reduction in acidic solution (Grashoff *et al.* 1983).

Results

Morphological differences

The basal holdfasts of individual plants were significantly heavier in dry weight (X= 1.549 $\pm$ 1.32, n=16) than the dry weight of the respective bladed region (X= 0.904 $\pm$ 0.802, n=16) (pval=0.004). The dry weight ratio of blade:base (g) ranged from 0.149 to 3.015, the average being 0.763 (Figure 2.2).

The size of the basal holdfast tended to depend on the substrate the alga was collected from, though there was no discernable pattern in the weight comparison of blade and base in this samples region. In sandy substrates, the basal holdfasts would reach deeper, and therefore be larger, while on rocky or other hard substrates, the base tended to be short and spread wider horizontally across the substrate. The blades on the other hand were variable at both substrates.

Localized nutrients in algal tissue

Avrainvillea samples showed a significant difference in concentrations of tissue N when comparing the basal holdfast and bladed regions (pval=0.00827) (Figure 2.2, Table A2). The bases had an average concentration of 8.381 μg N mg^{-1}, while the blades had an average concentration of 16.538 μg N mg^{-1}. There was also a significantly higher concentration of $\delta^{15}N$ in the basal holdfast (X = 1.02 $\pm$ 0.639, n=10) than the blades (X=0.538 $\pm$ 0.665, n=10) (pval=0.037). Site 10 had the lowest value of $\delta^{15}N$, at -0.4‰ for the basal holdfast and -0.5‰ for the blades (Figure 2.4).

Groundwater sample nutrients

Average concentration of nitrate and nitrite (N+N) for water samples taken close to algal patches was 1.00 µmol L^{-1} (+ 1.00, n=14) in contrast with 1.66 µmol L^{-1} (+ 1.10, n=14) for samples taken in sediment with no algae (Table A1). Ammonium concentrations for groundwater near algae patches and without algae were 2.38 µmol L^{-1} and 2.79 µmol L^{-1} respectively (p=0.057). Phosphorus, N+N, and ammonia concentrations are all slightly higher in locations without *Avrainvillea* than locations with *Avrainvillea*, though there are no significant differences.

Nearshore versus offshore groundwater

N+N concentrations in groundwater samples did not differ between nearshore (sites 1-8) and offshore sites (9-15) (Table A1). Average ammonium concentrations overall trended higher offshore (X=2.72 µmol L^{-1} $\pm$ 0.90, n=13) than nearshore (X =1.08 µmol L^{-1} $\pm$ 2.56, n=13), but were not statistically significant (pval =0.057).

Nearshore versus offshore algal tissue nutrients

Nearshore and offshore tissue nitrogen concentrations in basal holdfasts ranged from the lowest at 3.56 µgN mg^{-1} to 12.363 µgN mg^{-1} for nearshore and offshore samples, with no statistical differences between samples 1-15. There was, however, a significant difference in the concentration of nitrogen in the blades between nearshore (X = 17.72 $\pm$ 0.11, n=5) and offshore (X=19.65 $\pm$ 0.10, n=5) (pval=0.047).

Discussion

In the 1980's, Paikō Peninsula was a seagrass meadow (Atkinson 2007), and over a period of two decades, the invasive seaweed *Avrainvillea* grew quickly, and now covers the area with occasional other NIS. Such rapid growth strongly suggests significant nitrogen subsidies to sustain and maintain plant biomass.

In this exploratory survey of *Avrainvillea* in the Paikō area, the alga was found to draw down nutrients from the groundwater, and partition nitrogen. Due to its siphonous nature, *Avrainvillea* has no internal cell division, which is interesting given the clear partitioning of nutrients observed in this study. The lack of internal cell divisions could allow for nutrients to be housed equally throughout the alga, but there was a higher concentration of $\delta^{15}N$ in the basal holdfast while a higher concentration of tissue N was found in the bladed region (Figure 2.7). The average N concentration in the basal holdfast and blade nearshore samples was 1.0% and 1.4% respectively, while the offshore samples had an average concentration of 0.6% and 2.0%. The higher concentration of N in the bladed region compared to the basal holdfast could be a result of pulling nutrients from the water column, while the $\delta^{15}N$ values indicate drawing down nutrients from SGD (Figure 2.7). Therefore, the partitioning of nutrients may be a result of the location of algal tissue.

In areas with sewage pollution, $\delta^{15}N$ generally ranges from 7‰ to 38‰ (Dailer *et al.* 2010). Tissue $\delta^{15}N$ in this study was well below this, ranging from -0.05‰ to 1.8‰ at most, and likely represents the integration of nitrogen from all available sources over days or weeks (Dailer *et al.* 2010). These sources may include soil N, NH_4 in fertilizer and rain, and NO_3 fertilizer (Kendall and McDonnell 1998). The presence of a higher concentration of $\delta^{15}N$ in the basal holdfast may be a result of the way *Avrainvillea* collects nutrients from the groundwater or stores it for use in growth. Additionally, it is possible that similar to the species of *Avrainvillea* studied by Littler & Littler (1999), the blades may hold less nutrients because they are more likely to be abandoned in an adaptive attempt to control epiphyte presence on the blades. If the blades are easily lost, ecologically the nutrients would be more often saved when stored in the basal holdfast.

The base is proportionally larger than the blades on average and may grow faster or more than the upright blades, supporting the idea that the base is the apical region of this alga. Nutrients and photosynthesis are two key aspects to the growth of algae. Future research directions may benefit in exploring further how nutrients are drawn down into the basal holdfast versus the bladed region. Additionally, in algae cleanups, particular care should be taken to remove the entire basal holdfast from the sediment, especially as *Avrainvillea* has shown the ability to grow from basal fragments as small as 3 mm (Smith *et al.* 2002). Not only is the basal holdfast the more regenerative section of the alga, but the bigger basal holdfasts can leave behind more pieces for regeneration and continued growth if not removed carefully. As the holdfasts are sometimes attached to hard substrate, care should be taken when detaching the alga, to cause as little fragmentation and leave as few cells as possible.

Analysis of the concentration of inorganic nitrogen in the groundwater showed a tendency to be lower in places with algal patches versus those without. This suggests that *Avrainvillea* is drawing down groundwater fed porewater nutrients. Sites without obvious plants may still be lower than a true control because of the capacity for this alga to have undetected below-ground siphons. A larger sample size and larger patches may be needed to clarify this relationship. As the alga was less dense at offshore sites, this may point towards higher ammonia uptake at nearshore sites solely because the density is higher, or there may be a source of ammonia from SGD closer to the offshore sites. SGD has been shows to exist in gradients, with the higher levels of $\delta^{15}N$ nearshore and lower levels of $\delta^{15}N$ offshore (Amato *et al.* 2016). It is possible the density of *Avrainvillea* as well as the basal holdfast size is related to the existing nearshore – offshore gradients. Further, the cost of below-ground tissues may outweigh the benefit as the additional nutrients from SGD lessens, suggesting the size ratio of the basal holdfast may serve as an indicator for SGD. Further data is needed to confirm these possible relationships.

This study demonstrates *Avrainvillea*'s ability to collect and store nitrogen from the otherwise unaccessed nutrients in the groundwater, and the potential ecological advantages this gives the alga in Hawaiʻi. As this study was an initial step towards understanding the ecology of *Avrainvillea* in Hawaiʻi, further research should be conducted to confirm the trends discovered in this project. There is still much to study about *Avrainvillea* to understand its ecological success fully and become more successful in preventing its spread further in Oʻahu to preserve the future of Hawaii's reefs. Groundwater seeps are a major source of nutrients for algae, and likely the most effective step to take in halting the spread of *Avrainvillea* is to reduce nutrient runoff.

Another step in the control of *Avrainvillea* is the clearing of the invasive algae from affected areas. Knowing that the basal holdfast holds many nutrients and is the more regenerative part of the plant means that cleanup efforts should take extra care to remove the alga from its base and leave as small of fragments behind as possible. Not only should future research study the nutrient uptake pathways, but the life history of the alga as well. With terrestrial species, often the most effective way of controlling an invasive plant is to attack it at all life stages. Additionally, knowing the detailed characteristics of areas *Avrainvillea* has already invaded could potentially help identify other at-risk reefs and seagrass beds. With the spreading of

Avrainvillea around O‘ahu, the need to understand the alga has never been more important for the future of Hawaii's reefs.

Figure 2.1. (a-b) General location maps. (c) Site map of study location at Paikō Peninsula.

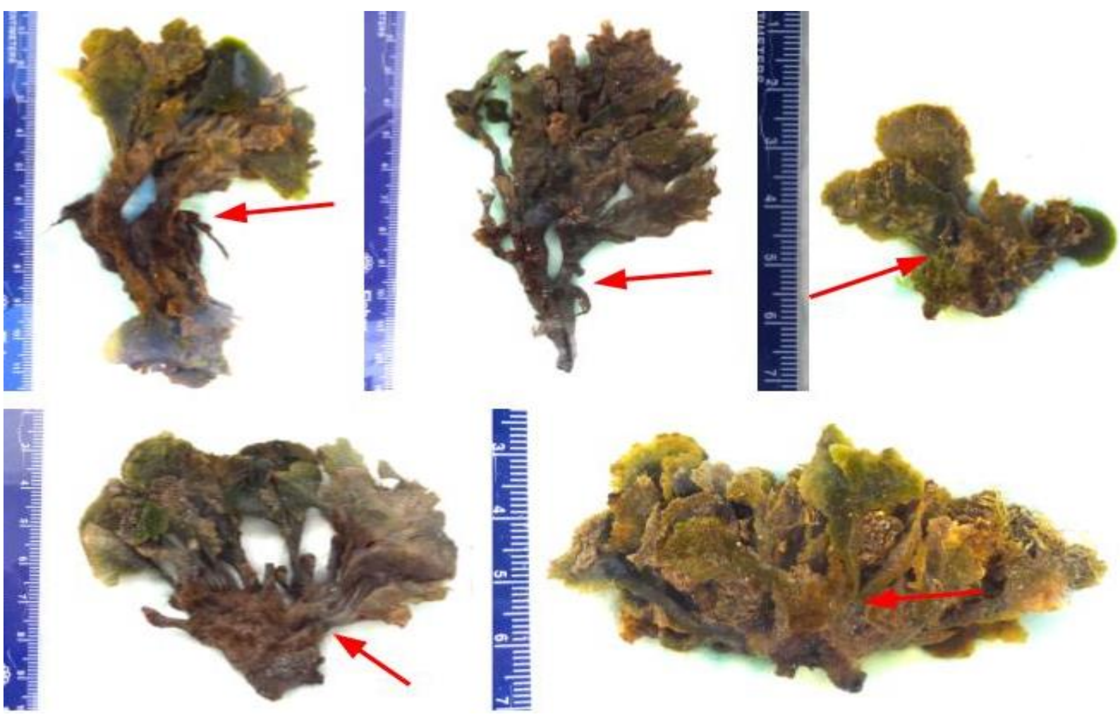

Figure 2.2. Specimens of *Avrainvillea* collected Oct 2017, from Maunalua Bay show a range of morphologies. The arrow highlights the point at which the stalks of the blade begin growing out from the basal holdfast.

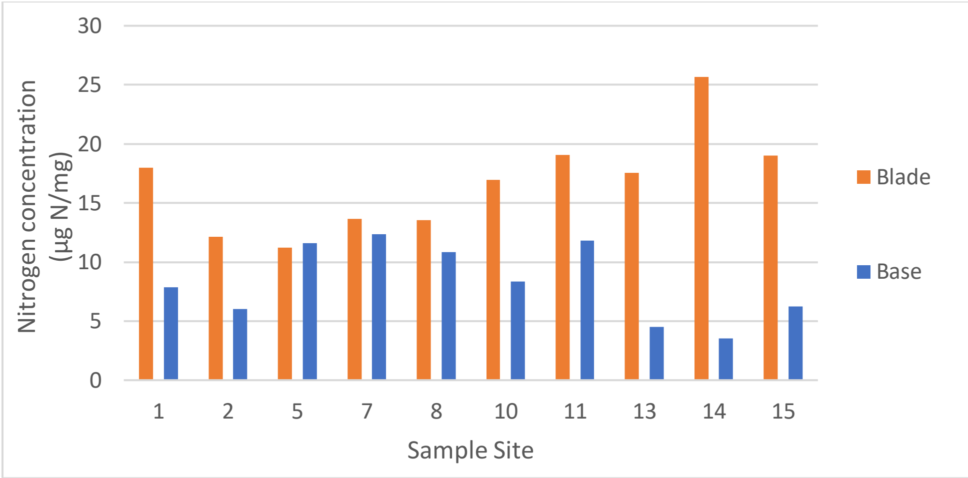

Figure 2.3. Comparison of the nitrogen concentration in tissue samples of *Avrainvillea* from the bladed region versus the basal holdfast at 10 sample sites.

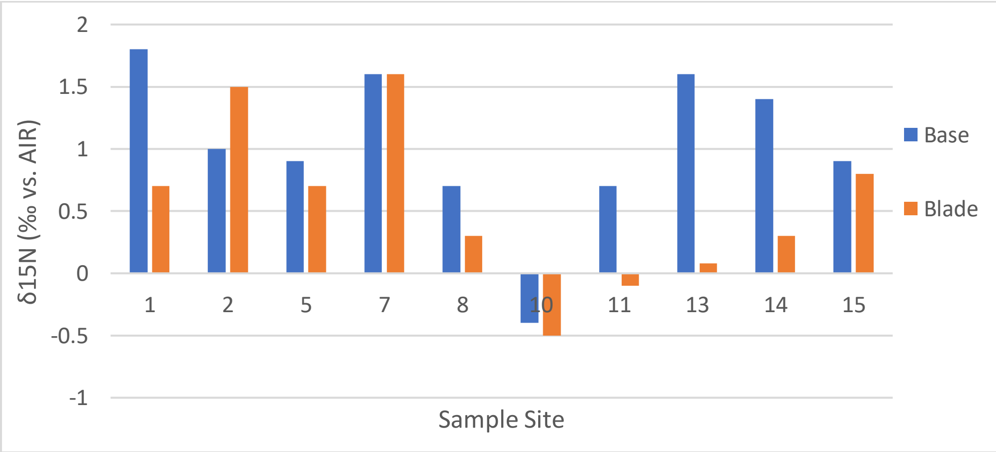

Figure 2.4. Individual values of δ^{15}N ‰ comparison of *Avrainvillea* basal holdfasts and the bladed regions at 10 sample sites.

Figure 2.5. (a) Mālama Maunalua's 'The Great Huki' – after 2 year algae cleanup (courtesy NOAA); (b) status in 2017. Lighter areas of sand in 2011 where the algae were systematically removed have all grown back and are once again covered by the darker algae in 2017.

Figure 2.6. A map of the site locations at Paikō, numbered 1-15. The length of the study site was approximately 400 meters along the shoreline and 170 meters offshore in water half a meter deep to water 1.5-2 meters deep respectively.

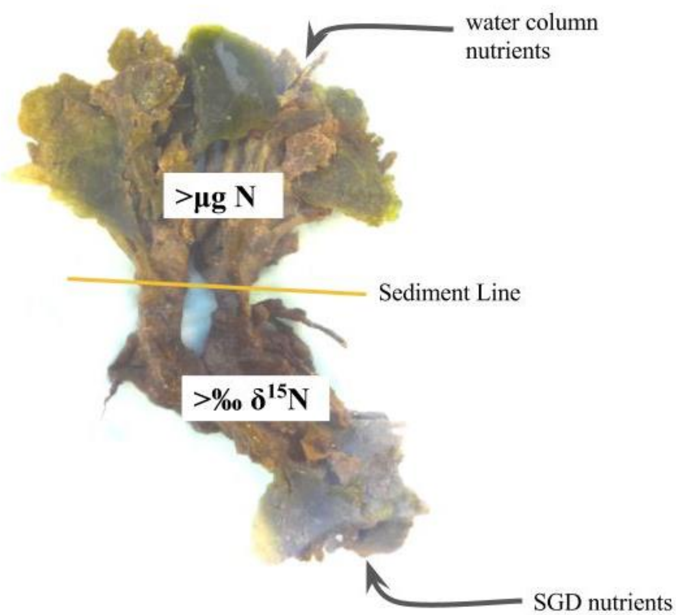

Figure 2.7. A conceptual model of the nutrient loading occurring within a single *Avrainvillea* specimen.

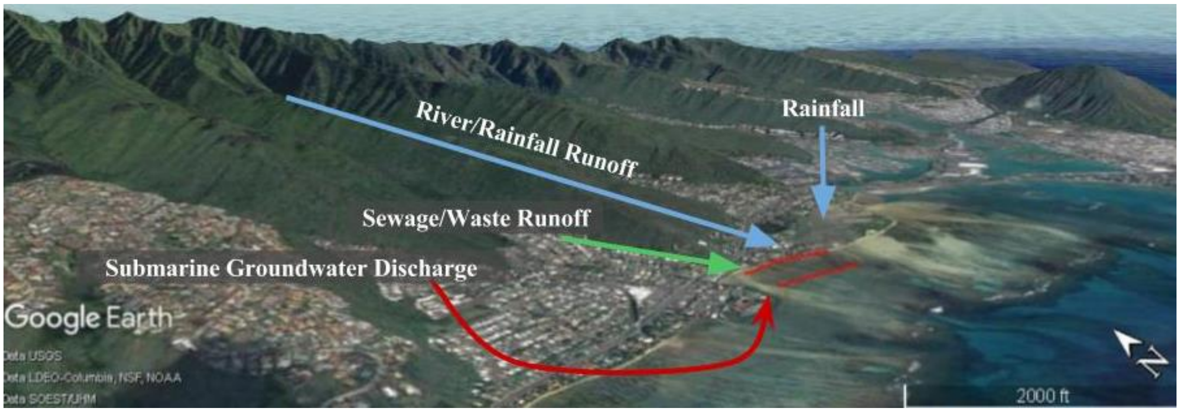

Figure 2.8. An overview of land-sea interactions and possible sources of nutrient loading in Maunalua Bay, which can cause phase-shifts from seagrass or coral cover to macroalgae dominance in nearshore reefs such as the reef at Paikō Peninsula.

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Mahalo nui loa to all

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Appendix A

Table A1. Dissolved inorganic nitrogen and phosphorus concentrations (µmol/L) taken from groundwater beneath algal patches and in sediment without algae as judged by % cover. Samples were collected at a depth of .5 m at nearshore sites and 1.5 m at offshore sites, approximately 5 cm into the sediment.

Site	Phosphate (algae)	Phosphate (no algae)	Silicate (algae)	Silicate (no algae)	N+N (algae)	N+N (no algae)	Ammonium (algae)	Ammoniu m (no algae)
1	1.35	0.59	23.83	9.80	0.49	0.35	8.77	1.30
2	0.47	0.54	5.32	8.80	0.30	3.47	2.29	1.57
3	0.85	0.64	7.30	12.95	1.06	1.60	2.67	2.01
5	0.60	0.70	18.76	18.76	0.44	2.70	1.81	3.25
6	0.42	0.46	5.65	12.34	0.50	2.99	1.32	0.55
7	0.55	0.91	16.75	20.03	0.45	2.20	1.06	1.99
8	0.62	0.35	13.91	18.47	0.61	0.63	5.23	0.71
9	0.53	0.45	12.15	5.62	3.46	2.38	7.41	3.69
10	0.16	0.13	2.13	3.60	0.26	0.28	0.53	1.23
11	0.15	0.13	3.83	3.25	0.30	2.49	1.14	1.43
12	0.11	0.41	1.65	13.97	1.14	0.43	0.86	8.12
13	0.32	0.24	2.99	4.95	1.29	0.54	2.63	2.99
14	0.56	0.77	3.52	6.28	2.72	0.69	1.88	8.09
15	0.37	0.25	8.57	1.55	0.39	1.12	2.17	0.65

Table A2. Tissue nitrogen concentrations for the blade region vs the basal holdfast of samples.

Sample Site	Base μg N	Base μgN/mg	Blade μg N	Blade μgN/mg	Base $\delta^{15}N$ (‰ vs. AIR)	Blade $\delta^{15}N$ (‰ vs. AIR)
1	40	7.88224	87.6	18.00765	1.8	0.7
2	29.1	6.03684	60.8	12.14809	1	1.5
5	59.6	11.613	53.9	11.20419	0.9	0.7
7	62.8	12.3634	69	13.67989	1.6	1.6
8	55.5	10.8577	67.8	13.56461	0.7	0.3
10	42.2	8.36223	82.7	16.95124	-0.4	-0.5
11	60	11.8339	94.8	19.06179	0.7	-0.1
13	22.7	4.5336	87.2	17.54174	1.6	0.08
14	17.5	3.5624	124	25.68245	1.4	0.3
15	32.1	6.2640	96.5	19.00766	0.9	0.8

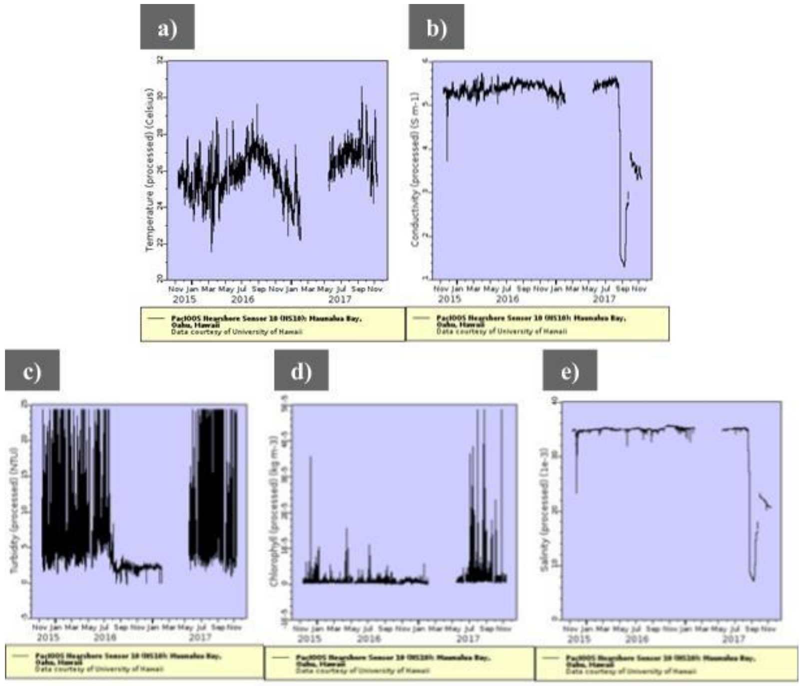

Figure A1. (a-f) Data records from Pacific Islands Ocean Observing System (PacIOOS) 2015-2017 measuring the temperature, conductivity, turbidity, chlorophyll, and salinity from Nearshore Sensor 10 (NS10), located off the old pier in Maunalua Bay in Hawaii Kai. The sensor is part of the PacIOOS and measure a variety of ocean parameters at fixed point locations along the south shore of O‘ahu. The instrument is a Sea-Bird Electronics model 16+ V2 coupled with a WET Labs ECO-FLNTUS combination sensor and is fixed to the pier at about 2m depth.

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